DIRECT IMMUNOFLUORESCENCE IN IMMUNOBULLOUS DISORDERS OF SKIN WITH HISTOPATHOLOGICAL CORRELATION

DISSERTATION

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PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH,

PEELAMEDU, COIMBATORE - 641 004

TAMILNADU, INDIA

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PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH
COIMBATORE

CERTIFICATE

This is to certify that the dissertation work entitled **"Direct Immunofluorescence in immunobullous disorders of skin with histopathological correlation"** submitted by Dr. Ram Ganesh V R, is work done by him during the period of study in this department from June 2007 to February 2010. This work was done under the guidance of Dr. Alamelu Jayaraman, Professor and Head, Department of Pathology, PSG IMS & R.

Dr. Alamelu Jayaraman
Professor and Head,
Department of Pathology,
PSG IMS & R.

Dr. S. Ramalingam
Principal,
PSG IMS & R.

Place:

Date:

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INTRODUCTION

There has been great progress during the past 5 decades in our understanding of the biology of the skin as it relates to the bullous diseases. One of the main reasons for continued identification of new bullous diseases is because diagnosis is based on immunological and molecular basis in addition to clinical and histological findings.

The stratified squamous epithelium of the human epidermis forms a continuous barrier against the external environment. The pathophysiology of bullous diseases illustrates how impairments in epithelial adhesion lead to disorders characterized by substantial morbidity and/or mortality. Blistering diseases can be inherited or acquired; most examples of the latter are autoimmune in nature and are characterized by autoantibodies that target junctional molecules promoting either cell-cell or cell-matrix adhesion in skin. This latter group together constitutes the spectrum of immunobullous disorders.

The pemphigus group of diseases is associated with antibodies to desmosomal proteins. The auto-antibody in each type of pemphigus is directed against a unique desmososmal protein or a combination of desmosomal proteins. The subepidermal bullous diseases are associated with antibodies against one or more components of the Basement Membrane Zone (BMZ).

The diagnostic specificities of the clinical and histopathological findings vary among the bullous diseases with significant overlap between the various entities. The commonly used modalities for the diagnosis of immunobullous disorders are histopathology and immunofluorescence (both Direct and Indirect). In addition, higher centres employ techniques such as ELISA, immunoblotting and immunoelectron microscopy which give highly accurate results (used more often for research).

Direct Immunofluoresence(DIF) helps detect molecules such as antibodies and complement components within biopsy specimens taken from perilesional skin.

The differential diagnosis of a DIF test depends on four features:

1. Primary site of immune deposition
2. Class of Immunoglobin or other type of immune deposit
3. If multiple, the identity of the most intense deposits
4. Deposition in other sites other than the main site

These parameters help attain a pattern approach which would in turn complement clinical and histological examination. Thus, DIF has time and again proven to be an asset in the diagnosis of the spectrum of immunobullous diseases.

Although the use of immunofluorescence in the diagnosis of immunobullous lesions has shown enormous progress in Western countries, in India its use is restricted to a few centers. This study aims at estimating the proportion of immunobullous disorders among the total number of skin lesions that required biopsy at our institution, and also comparing histopathology and direct immunofluorescence findings of these cases. These findings would also contribute towards defining the role of DIF in the diagnosis of immunobullous lesions of skin and would provide a base for further studies in this area.

AIMS AND OBJECTIVES

1. To estimate the proportion of immunobullous lesions among the total number of skin biopsy samples examined at a tertiary care center over a period of two years.

2. To study and interpret the staining pattern of various types of immunobullous lesions with direct immunofluorescence.

3. To correlate direct immunofluorescence findings with histopathology in immunobullous lesions of skin.

MATERIALS AND METHODS

Patients with bullous lesions of skin, who visited the Department of Dermatology, PSG Hospitals, Coimbatore between Aug ’07 and Aug ’09, for whom both direct immunofluorescence and histopathology had been requested with adequate and properly stored biopsies, were included in this study.

SAMPLE COLLECTION:

After evaluating the patient clinically, two punch biopsies of atleast 0.3 cm diameter were taken from each patient. One specimen was fixed in 10% formalin for routine histopathology and the other was preserved in Michel’s medium for immunofluorescence study. Both containers were labeled appropriately and dispatched to the lab.

MATERIALS USED:

1. Spirit swab to clean the skin
2. 2% xylocaine for local anaesthesia
3. 10% formalin
4. Michel’s medium for immunofluorescence
5. Cryostat
6. Fluorescent conjugated antibody
7. Fluorescent microscope using UV light with suitable filters

HISTOPATHOLOGY:

The punch biopsy taken from an early lesion with surrounding skin tissue (sent in formalin) was processed in an automated tissue processing unit before being embedded in paraffin and taken for sectioning. The sections were stained using routine Hematoxylin & eosin, mounted and labeled.

DIRECT IMMUNOFLUORESCENCE:

DIF is a technique used to detect the presence of immunoreactants deposited in vivo in the patient's skin or mucosa. The immuoreactants detected include immunoglobulins, components of the complement system and fibrinogen.

This technique uses fluorochrome dyes tagged with anti-human immunoglobulin. They bind to the specific immunoreactant already bound to epitopes within the epidermis. After washing to ensure removal of the excess dye, the tissue is viewed under UV light. The fluorochrome has the property to emit incident UV light as fluorescent radiation, thereby allowing visual localization of the immunoreactant in question under the microscope. The colour of the fluorescent radiation depends on the dye used.

The most widely used fluorochrome is Fluorescein. It is best conjugated to protein in the form of fluorescein isothiocyanate which emits an apple green fluorescence.

PROCEDURE:

For DIF, biopsy specimens were taken from the perilesional skin and sent in Michel's medium (transport medium).

Composition of Michel's medium:

- Solution A:

1 M potassium/ sodium citrate buffer (pH 7.0)	2.5 mL
0.1 M Magnesium sulphate	5.0 mL
0.1 M ethylmelenide	5.0 mL
Distilled water	87.5 mL

- MICHEL'S MEDIUM:

Solution A	100 mL
Ammonium sulphate	55 gm

Adjust to pH 7.2

i. The biopsy was snap frozen in the cryostat at -18˚C using OCT compound as the freezing medium

ii. Sections of 4 micron thickness were cut with atleast 2 sections taken on each slide.

iii. Five slides were made for each biopsy

iv. The sections were then dried under an electric fan for 10 mins

v. They were then washed in phosphate buffered saline (pH 7.1-7.2)

vi. The sections were then dried under an electric fan for 10 mins

vii. The slides were covered with one of the following FITC labeled anti-sera:

A. IgG diluted 1:200 in PBS

B. IgM diluted 1:100 in PBS

C. IgA diluted 1:100 in PBS

D. C 3 diluted 1:100 in PBS

E. Fibrinogen diluted 1:200 in PBS

viii. The slides were incubated with anti-sera in a moist chamber at 37˚C for 30 mins

ix. The sections were washed thrice in phosphate buffered saline.

x. They were then dried under an electric fan for 10 mins

xi. The sections were mounted using buffered glycerol and examined using Leica DM1000 fluorescent microscope with HG 50 UV lamp and a blue filter.

The immunofluorescence pattern was studied and positivity was graded arbitarily as strongly positive (+++), moderately strong (++) and weak deposits (+).

The remains of the tissue after the sections were taken, was wrapped in an aluminium foil (along with embedding medium) and stored in a freezer at -20˚C till the end of the study period.

REVIEW OF LITERATURE

HISTOLOGY OF SKIN

Skin is divided into two seemingly separate but functionally interdependent layers (epidermis and dermis), and composed of cells with myriad functions ranging from mechanical and photoprotection, immunosurveillance, nutrient metabolism, and repair.

The epidermal layer is composed primarily of keratinocytes (>90%), with minority populations of Langerhans cells, melanocytes, neuroendocrine (Merkel) cells, and unmyelinated axons. Architecturally, the epidermal layer has an undulant undersurface in two-dimensional sections, with downward invaginations termed rete, and interdigitating mesenchymal cones termed dermal papillae.

The dermis is separated from the epidermis by a structurally and chemically complex basement membrane zone. The constituents of the dermis include endothelial and neural cells, supporting elements, fibroblasts, dendritic and nondendritic monocyte/macrophages, factor XIIIa-expressing dermal dendrocytes, and mast cells enveloped within a matrix of collagen and glycosaminoglycan.

Adnexae extend from the epidermis into the dermis and consist of specialized cells for hair growth, epithelial renewal (stem cells), and temperature regulation. The subcutis is an underlying cushion formed by cells engorged with lipid and nourished by vessels that grow within thin intervening septae.

EPIDERMIS:

Two types of cells constitute the epidermis: keratinocytes and dendritic cells. The keratinocytes differ from the dendritic cells, or clear cells, by possessing intercellular bridges and ample amounts of stainable cytoplasm. As they differentiate into horny cells, the keratinocytes are arranged in four layers: basal cell layer (stratum basalis), squamous cell layer (stratum spinosum), granular layer (stratum granulosum), and horny layer (stratum corneum)

The terms stratum malpighii and rete malpighii are often applied to the three lower layers, which contain the basal, squamous, and granular cells, and comprise the nucleated, viable epidermis. An additional layer, the stratum lucidum, can be recognized in areas having a thick stratum granulosum and corneum forming the lowest portion of the horny layer, especially on the palms and soles.

INTERCELLULAR JUNCTIONS:

The cells are separated by spaces that are traversed by intercellular bridges. The tonofilaments within the cytoplasm of the keratinocytes of the stratum spinosum are loose bundles of electron-dense filaments, each filament measuring 7 to 8 nm in diameter. The tonofilaments at one end are attached to the attachment plaque of a desmosome, and the other end lies free in the cytoplasm near the nucleus. The desmosomes correlate with the intercellular bridges. Each desmosome possesses two electron-dense attachment plaques, one at either end, that are located in the cytoplasm of the two keratinocytes which the desmosome connects. Next to each attachment plaque lies the trilaminar plasma membrane of the two keratinocytes.

In the center of the desmosome lies the intercellular cement substance, referred to also as glycocalyx, containing glycoproteins. Desmosomal cadherins are desmogleins and desmocollins that localize to desmosomes and are linked to intracytoplasmic intermediate filaments by plakoglobin and desmoplakin. Within the desmosome complex, desmogleins within the cell membrane bind to plakoglobin through their cytoplasmic domain. Intermediate keratin filaments anchor at the desmosomal plaque, possibly by way of the carboxy-terminal domain of plakoglobin.

Desmoglein 3 is normally concentrated between immediately suprabasal keratinocytes, while desmoglein 1 is distributed principally among keratinocytes directly beneath the stratum corneum.

DERMO-EPIDERMAL JUNCTION:

A subepidermal basement membrane zone not visible in sections stained with hematoxylin-eosin is seen on staining with the PAS stain. It appears as a homogeneous band. The light microscopic PAS-positive basement membrane zone is on the average 20 times thicker than the electron

microscopic basement membrane, or lamina densa. A basement membrane zone similar to that seen at the epidermal-dermal border is present also around the cutaneous appendages.

Proceeding from the epidermis to the dermis, there are four distinct structural components of the epidermal basement membrane which have been identified based on electron microscopy[10,12] :

- Intermediate filament, hemidesmosomal plaques, and plasma membranes of the basal keratinocytes with Bullous pemphigoid antigen (BPA)
- Electron-lucent Lamina lucida that contains delicate anchoring filaments connecting hemidesmosomes in basal keratinocytes to the underlying lamina densa
- Electron-dense Lamina densa that provides the basement membrane with much of its strength. The main component is type IV collagen[10,12]
- Sublaminar densa region containing anchoring fibrils (type VII collagen), anchoring plaques, and filamentous proteins of the papillary dermis

Hemidesmosomes contain bullous pemphigoid antigen 1 (BPAG1; 230 kD), bullous pemphigoid antigen 2 (BPAG2; 180 kD), integrin alpha, and other molecules.

IMMUNOBULLOUS DISEASES:

Immunobullous skin diseases are a group of autoimmune blistering diseases that affect skin and mucous membranes and are caused by or associated with the deposition of specific antibodies on cutaneous structures.

A blister is defined as a fluid filled cavity formed within or beneath the epidermis. The fluid consists of tissue fluid and plasma. A variable component of inflammatory cells may also be present.

One useful distinction is the categorization of blisters into vesicles (blisters <0.5 cm in diameter) and bullae (blisters >0.5 cm in diameter). For example, vesicles characteristically occur in

dermatitis herpetiformis as opposed to pemphigoid, in which bullae are most commonly observed.

MECHANISMS OF BLISTER FORMATION:

SPONGIOSIS:

Spongiosis is the accumulation of extracellular fluid within the epidermis with resultant separation of the keratinocytes. Pronounced spongiosis leads to disruption of desmosomes and subsequent blister formation. The increasing accumulation of fluid leads to a vesicle and, in some instances, to a bulla. Marked spongiosis may terminate in reticular degeneration. Spongiosis is almost always associated with an infiltrate of lymphocytes within the epidermis and around superficial vessels. However, spongiosis is a passive event associated with increased permeability of the superficial vascular plexus, particularly the postcapillary venules.

ACANTHOLYSIS:

Acantholysis results from the loss of appropriate keratinocyte cell-cell contact. Histologic evidence of acantholysis includes the presence of rounded keratinocytes with condensed cytoplasm and large nuclei with peripheral condensation of chromatin and prominent nucleoli.

RETICULAR DEGENERATION:

Reticular degeneration results from ballooning degeneration (intracellular edema) with secondary rupture of the keratinocytes. The remaining desmosomal attachments often connect strands of ruptured keratinocytic membranes and cytoplasm to intact keratinocytes, giving the epidermis an irregular meshwork appearance.

CYTOLYSIS:

Cytolysis is the disruption of keratinocytes. It occurs in the normal epidermis when the structural (keratin) matrix and desmosomal plaques of the keratinocyte are overwhelmed by high levels of physical agents such as friction and heat. Minimal friction may lead to cytolysis in subjects whose keratinocytes do not have normal structural matrix and desmosomes, such as in epidermolysis bullosa

simplex and epidermolysis bullosa of the Cockayne-Weber type.

BASEMENT MEMBRANE ZONE DISRUPTION:

Basement membrane zone disruption or destruction results from primary structural deficiencies as well as from both humoral and cellular immunologically mediated damage. When blisters arise at the epidermal basement membrane zone, any of the specific subanatomic compartments can be affected.

CLASSIFICATION OF IMMUNOBULLOUS DISORDERS:

INTRA-EPIDERMAL BLISTERS:

SUB-CORNEAL BLISTERING DISORDERS:

- Pemphigus foliaceous
- Pemphigus erythematosus
- Intra-epidermal neutrophilic IgA dermatoses
- Herpetiform pemphigus

SUPRA-BASAL BLISTERING DISORDERS:

- Pemphigus vulgaris
- Pemphigus vegetans
- Paraneoplastic pemphigus

SUB-EPIDERMAL BLISTERING DISORDERS:

- Bullous pemphigoid
- Cicatrical pemphigoid
- Pemphigoid gestationis
- Epidermolysis bullosa acquistica
- Bullous SLE
- Dermatitis herpetiformis

- Linear IgA bullous dermatoses

INTRA-EPIDERMAL AUTOIMMUNE BLISTERING DISEASES

PEMPHIGUS

Pemphigus is a rare vesico-bullous disease having a grave prognosis. Acantholytic cells and clefts or bullae are formed in the epidermis as a result of interaction of antibodies with epidermal intercellular cement substance. In 1880, Auspitz recorded the histological findings of pemphigus blister and coined the term acantholysis[2].

Lever has classified pemphigus into two categories based on the level of the blister. Pemphigus vegetans is considered a variant of pemphigus vulgaris with suprabasal clefts. Pemphigus erythematosus was considered a variant of pemphigus foliaceous due to a subcorneal bulla.

AUTOANTIBODIES:

The pathogenesis of pemphigus is due to presence of circulating autoantibodies against the cell-membrane components of keratinocytes important in cell-cell adhesion. These antibodies are demonstrable by direct immunofluorescence (DIF) testing of skin, and indirect immunofluorescence (IIF) testing of serum.

Pemphigus can be divided into five types: (a) pemphigus vulgaris, with its reactive state, pemphigus vegetans; (b) pemphigus foliaceus, with its lupus-like variant, pemphigus erythematosus, and its endemic variant, fogo selvagem; (c) drug-induced pemphigus; (d) IgA pemphigus; and (e) paraneoplastic pemphigus.

TARGET ANTIGENS IN PEMPHIGUS

Diseases	Auto-antibodies	Antigens	Location of Antigens
Pemphigus vulgaris			
Mucosal mainly	IgG	Desmoglein 3 (130 kD)	Desmosomes
Mucocutaneous	IgG	Desmoglein 3 (130 kD)	
		Desmoglein 1 (160 kD)	
Pemphigus foliaceus	IgG	Desmoglein 1 (160 kD)	Desmosomes
Paraneoplastic pemphigus	IgG	Desmoglein 1 (160 kD)	Desmosomes or hemidesmosomes
		Desmoglein 3 (130 kD)	
		Desmoplakin I (250 kD)	
		plakoglobin (82 kD)	
Drug-induced pemphigus	IgG	Desmoglein 3 (130 kD)	Desmosomes
		Desmoglein 1(160 kD)	
IgA pemphigus			
SPD type	IgA	Desmocollin 1 (110/100 kD)	Desmosomes
IEN type	IgA	Desmoglein 1 (160 kD)	
		Desmoglein 3 (130 kD)	

SPD – Subcorneal pustular dermatosis; IEN – Intra-epidermal neutrophilic dermatosis

CLINICAL FEATURES:

Numerous small flaccid bullae are present on the scalp and trunk in pemphigus vulgaris. The disease is characterized by thin-walled, flaccid bullae which rupture to leave slow-healing erosions. The site of onset is frequently the oral mucosa with upto two-thirds of patients developing oral lesions as the site of initial involvement. The disease may remain localized to the oral mucosa for months before eventual progression[38].

Pemphigus vegetans is much less common and is characterized by inter-triginous verrucous plaques. Paraneoplastic pemphigus presents with oral and cutaneous erosions and bullae in patients with an underlying neoplasm, usually lymphoma.

PEMPHIGUS VULGARIS:

HISTOPATHOLOGY:

It is important that early blisters, preferably small ones, are selected for biopsy. If no recent blister is available, an old one may be moved into the neighboring skin by gentle vertical pressure with a finger (positive Nikolsky sign).

The earliest recognized change may be either rare eosinophilic spongiosis in the lower epidermis. Acantholysis leads first to the formation of clefts, and then to blisters in a predominantly suprabasal location. The intraepithelial acantholysis may extend into adnexal structures, or occasionally be higher in the stratum spinosum. The basal keratinocytes, although separated from one another through the loss of attachment, remain firmly attached to the dermis like a 'row of tombstones'. Within the blister cavity, the acantholytic keratinocytes, singularly or in clusters, have rounded, condensed cytoplasm about an enlarged nucleus with peripherally palisaded chromatin and enlarged nucleoli. Recent studies[15] seem to indicate that certain proteolytic enzymes, such as plasminogen activators may be responsible for the acantholytic process.

There is little inflammation in the early phase of blister formation. If present, it is usually a sparse, lymphocytic perivascular infiltrate accompanied by dermal edema. If, however, eosinophilic spongiosis is apparent, numerous eosinophils may infiltrate the dermis. The phenomenon of eosinophilic spongiosis occurs occasionally in other blistering diseases, particularly in their early phases, including acute contact dermatitis, pemphigus foliaceus, bullous pemphigoid, herpes gestationis, drug eruptions, spongiotic arthropod bite reactions, and transient acantholytic dermatosis.

Several important changes ensue as the lesions age. First, a mixed inflammatory cell reaction consisting of neutrophils, lymphocytes, macrophages, and eosinophils may develop. Because of the instability of the blister roof, erosion and ulceration may occur. Older blisters may also have several

layers of keratinocytes at the blister base because of keratinocyte migration and proliferation. Lastly, there may be considerable downward growth of epidermal strands, giving rise to so-called villi.

IMMUNOFLUORESCENCE FINDINGS:

Beutner and Jordan[30] first demonstrated immunoglobulin in skin lesions and sera of patients suffering from pemphigus. The edge of a blister with intact surrounding normal skin or uninvolved skin adjacent to a blister should be supplied for study[18]. The tissue may be snap-frozen or transported in Michel's medium. DIF testing is a very reliable and sensitive diagnostic test for pemphigus vulgaris, in that it demonstrates IgG in the squamous intercellular/cell surface areas ('fish-net' appearance) in up to 95% of cases, including early cases and those with very few lesions, and in up to 100% of cases with active disease. It remains positive often for many years after clinical disease has regressed.[7] Cormane and Chorzelski (1967)[56] demonstrated participation of complement at the same site. Negative DIF findings when the patient is in remission may be a good prognostic indicator. Fluorescence may also be observed at the dermo-epidermal junction (linear BMZ deposits) rarely as observed by Judd and Lever[30]. Beutner et al[30] demonstrated such occurrence primarily in lesions of the face and other sun-exposed areas.

Unfixed frozen sections of guinea pig esophagus, monkey esophagus, or normal human skin are used as substrate for indirect IF testing. In general, monkey esophagus is the best substrate for IIF studies[14]. IgG is demonstrated in the squamous intercellular substance in 80% to 90% of cases, and the titer correlates with disease activity. False-positive indirect tests can occur as antidesmoglein autoantibodies are sometimes found in patients with no bullous disease, also known by some authors as 'pseudo-pemphigus'[19]. For example, they have been found in patients with silicosis, and in relatives of patients with pemphigus vulgaris.

PEMPHIGUS VEGETANS:

Pemphigus vegetans is an uncommon chronic variant of pemphigus vulgaris, comprising only 1% to 2% of the cases. The disease has a better prognosis than pemphigus vulgaris with occasional cases showing spontaneous remission. [41]

TYPES:

- Neumann type: the disease begins and ends as pemphigus vulgaris, but many of the denuded areas heal with verrucous vegetations that may contain small pustules in early stages
- Hallopeau type: is relatively benign, having pustules as the primary lesions instead of bullae. Their development is followed by the formation of gradually enlarging verrucous vegetations, especially in intertriginous areas

HISTOPATHOLOGY:

In the Neumann type, the early lesions consist of bullae and denuded areas that have the same histologic picture as that of pemphigus vulgaris. As the lesions age, however, there is formation of villi and verrucous epidermal hyperplasia. Numerous eosinophils are present within the epidermis and dermis, producing both eosinophilic spongiosis and eosinophilic pustules. Acantholysis may not be present in older lesions.[2,10]

In the Hallopeau type, the early lesions consist of pustules arising on normal skin with acantholysis and formation of small clefts, many in a suprabasal location. The clefts are filled with numerous eosinophils and degenerate acantholytic epidermal cells. Early lesions may reveal more eosinophilic abscesses than in the Neumann type. The subsequent verrucous lesions are histologically identical to the Neumann type.

IMMUNOFLUORESCENCE FINDINGS:

DIF examination reveals squamous intercellular IgG in all reported cases.

PEMPHIGUS FOLIACEOUS:

The entity pemphigus foliaceous was described by Cazenave in 1844[47]. Brazilian pemphigus foliaceous or fogo selvagem is an endemic form of pemphigus foliceous seen in Brazil. The cause is unknown but farmers bit by the black fly (Simulium pruinosum) are predisposed to developing the disease.[10]

The blisters in pemphigus foliaceous rupture rapidly and are often not seen. Painful, crusted, offensive and inflamed erosions involve the central face, scalp, chest and upper back and may evolve into an erythroderma[19]. Mucosal involvement is rare[41]. Itching is usually a significant complaint.[50]

HISTOPATHOLOGY:

The earliest change consists of acantholysis in the upper epidermis, within or adjacent to the granular layer, leading to a subcorneal bulla. The number of acantholytic keratinocytes is usually small, often requiring a careful search to identify them. Secondary clefts may develop, leading to detachment of the epidermis in its mid level.

In the setting of a subcorneal blister, dyskeratotic granular keratinocytes are diagnostic for this disorder.[10] Eosinophilic spongiosis may be prominent with intraepidermal eosinophilic pustules. The character of the inflammatory infiltrate is variable.

IMMUNOFLUORESCENCE FINDINGS:

DIF testing of perilesional skin is positive in the vast majority of cases. Two patterns of pemphigus antibody deposition have been described. In most cases, there is full-thickness squamous intercellular substance deposition of IgG which can be indistinguishable from pemphigus vulgaris[19]. IgG may also be localized only to the superficial portion of the epidermis.

PEMPHIGUS ERYTHEMATOSUS:

In 1926, Senear and Usher described pemphigus erythematosus as a variant of pemphigus foliaceous with some clinical and immunological features of lupus erythematosus.[37] Other than lupus erythematosus, it has also been associated with other diseases like thymoma, bronchogenic carcinoma and myasthenia gravis as well as some drugs like pencillamine.

HISTOPATHOLOGY:

The light microscopic features are identical to those of pemphigus foliaceus. Interface dermatitis may also be apparent in rare cases, making distinction from lupus erythematosus difficult. The blister may contain numerous neutrophils, which can make distinction from subcorneal pustular disorders especially difficult[41].

IMMUNOFLUORESCENCE FINDINGS:

DIF testing of perilesional skin reveals squamous intercellular substance deposition of IgG in greater than 75% of cases, as well as linear or granular deposition of IgG and/or complement (i.e., a positive lupus band test) at the dermo-epidermal junction. Typically the latter deposits are over the sun-exposed regions of the skin but may also involve the non-sun-exposed areas[41].

PEMPHIGUS HERPETIFORMIS

Pemphigus herpetiformis is a variant of pemphigus vulgaris with clinical features resembling dermatitis herpetiformis. The sexes are affected equally and the disease affects a wide age group (31 to 83 years).

Patients present with intensely pruritic, grouped, erythematous papules, vesicles and blisters, rarely associated with involvement of mucous membranes. There is a tendency to involve the extensor surfaces of the extremities.

HISTOPATHOLOGY:

The biopsy findings are variable and non-specific. Eosinophilic spongiosis, intra-epidermal vesicles and pustules of variable composition have been described. Acantholytic cells are usually identified.

IMMUNOFLUORESCENCE FINDINGS:

Immunofluorescence shows IgG in an intercellular pattern characteristic of the pemphigus group. In most patients, Dsg1 is the target antigen with few showing antibodies against Dsg3.

IgA PEMPHIGUS:

IgA pemphigus is a dapsone responsive variant of pemphigus that is characterized clinically by pustular rather than bullous or vesicular lesions. Most patients are middle aged or elderly but children may also be affected. Sex incidence is equal. There is an association with malignant monoclonal IgA gammopathies[19].

IgA pemphigus is divided into two major subtypes: sub-corneal pustular dermatosis variant (SPD) and Intra-epidermal neutrophilic IgA dermatosis variant (IEN).

Patients with SPD-like variant present with superficial, flaccid pustular lesions with erythematous base arising over the trunk and proximal extremities.

Patients with the IEN IgA dermatosis present with generalized pustules and crusts with peripheral vesicles (sun-flower configuration).

HISTOPATHOLOGY:

(I) SPD VARIANT:

Lesions show subcorneal vesicles associated with a neutrophilic infiltrate. The presence of neutrophils may be due to the presence of IgA since IgA is associated with neutrophil chemotaxis.

(II) IEN VARIANT:

Biopsy shows pustules distributed throughout all layers of the epidermis with involvement of the hair follicle. Acantholytic cells are usually present.

IMMUNOFLUORESCENCE FINDINGS:

SPD IgA pemphigus shows intercellular IgA deposition in the upper epidermis while the IEN variant shows the antibodies deposited preferentially in the lower epidermis. In some patients, the entire thickness of the epidermis may show IgA deposition.

PARANEOPLASTIC PEMPHIGUS:

Paraneoplastic pemphigus is a variant of pemphigus which may be associated with a wide range of tumors. Lymphoma is most often the coexistent neoplasm[19,41].

Patients present with refractory, painful, persistent erosions of the oral mucosa and lips. Skin lesions are characterized by polymorphic, pruritic papulosquamous dermatoses with subsequent blistering involving the trunk, proximal extremities and target lesions over the palms and soles[19]. The disease is associated with a very high mortality.

HISTOPATHOLOGY:

The lesions show an admixture of suprabasal acantholysis, basal cell liquefactive degeneration, dyskeratotic keratinocytes and lymphocytic exocytosis. A lichenoid chronic inflammatory cell infiltrate is typically seen in the superficial dermis.

IMMUNOFLUORESCENCE FINDINGS:

Direct immunofluorescence shows IgG deposition affecting the whole thickness of the epidermis whereas C3 is seen only in the lower layers. Characteristically, the staining is focal and faint[41]. Rat bladder transitional epithelium is a specific and sensitive substrate for Indirect immunofluorescence[19].

SUB-EPIDERMAL BULLOUS DISEASES

Subepidermal bullous diseases are disorders in which a blister forms along the dermo-epidermal junction. This group of diseases includes conditions with different clinical presentations, histologic findings, and pathogenesis. Both inherited and acquired alterations in key adhesion proteins at or in the dermo-epidermal junction result in blister formation.

TARGETS COMMON TO AUTOIMMUNE AND INHERITED BLISTERING DISEASES

Protein Target	**Structural Target**	**Autoimmune Disease**	**Genetic Disease**
BPAG1 (BP230)	HD	BP	None identified
BPAG2 (BP180, type VII collagen)	HD-anchoring filament complexes	BP, PG, CP, LABD	GABEB
α4 integrin	HD-anchoring filament complexes	Ocular CP	Junctional EB with pyloric atresia
Laminin 5	Lamina lucida-lamina densa interface	Antiepiligrin CP	Junctional EB-Herlitz
Type VII collagen	Anchoring fibrils	EB acquisita, bullous systemic lupus erythematosus	Dystrophic EB

BP, bullous pemphigoid; CP, cicatricial pemphigoid; EB, epidermolysis bullosa; HD, hemidesmosome; PG, pemphigoid gestationis; LABD, linear IgA bullous dermatosis. GABEB, generalized atrophic benign epidermolysis bullosa, BPAG, bullous pemphigoid antigen; HD, hemisdesmosome

BULLOUS PEMPHIGOID

First described in 1953[10], bullous pemphigoid is an autoimmune bullous disorder which affects primarily elderly patients[39,42]. It is uncommon in children[42] with a higher propensity for involvement of the oral mucosa and face when compared to adults. Clinically and histologically, childhood disease is often confused with chronic bullous dermatosis of childhood[41].

CLINICAL FEATURES:

The course is chronic and benign. In contrast to pemphigus, the Nikolsky sign is negative. The lesions involve the trunk, flexor surfaces of extremities and intertriginous areas, with the oral mucosa involved in about one third of the cases. Vulval lesions can mimic eruptions of herpes simplex[16]. Bullous pemphigoid may start as a nonspecific eruption suggestive of urticaria or dermatitis, and can persist for weeks or months. Most patients have high serum IgE levels which appear to be related to disease activity[3].

HISTOPATHOLOGY:

In early lesions, papillary dermal edema in combination with a cell-poor or cell-rich perivascular lymphocytic and eosinophilic infiltrate is present. The blister arises at the dermo-epidermal junction. In the cell-rich pattern, the blisters arise on erythematous skin. Eosinophilic papillary abscesses may develop with numerous perivascular and interstitial eosinophils intermingled with lymphocytes and neutrophils in the superficial and deep dermis. Eosinophilic spongiosis may occur. The cell-poor pattern is observed when blisters develop on relatively normal skin, in which there is usually a scant perivascular lymphocytic infiltrate with few eosinophils. The blister lumen contains few inflammatory cells. Epithelial migration and regeneration may result in an intraepidermal location in older blisters. Similar to pemphigus vegetans, a pseudocarcinomatous hyperplasia of the epidermis, subepidermal bullae, and accumulations of eosinophils and lymphocytes may be seen[10].

IMMUNOFLUORESCENCE TESTING:

DIF testing of perilesional skin has shown linear C3 deposition at the dermo-epidermal junction in virtually 100% of cases and IgG in 65% to 95%. The antibodies are predominantly IgG1 and IgG4 subclasses[19]. Similarly deposited IgA and IgM are observed in about 25% of cases. No correlation exists between the antibody titre and the clinical severity of the disease. The IgG is located within the lamina lucida, where it appears bound specifically to the hemidesmosomes.

Linear deposits of IgG and C3 at the basement membrane zone are typical of, but not specific for bullous pemphigoid[29]. A similar pattern is also found in cicatrical pemphigoid, pemphigoid gestationis, epidermolysis bullosa acquisita and bullous SLE as serum anti-basement membrane zone antibodies are found in these patients also[14,34,35,39].

Thus three types of investigations have been proposed for an accurate diagnosis of bullous pemphigoid[35]:

(i) Direct immunoelectron microscopy

(ii) Immunofluorescence on salt split skin

(iii) Immunoblotting technique

Immunoelectron microscopy (IEM) and immunochemical methods have demonstrated that Bullous pemphigoid and Epidermolysis bullosa aquisita are immunologically distinct diseases. Western immunoblots have shown that BP autoantibodies recognize a 230 kDa or 180 kDa[25] epidermal protein associated with hemidesmosomes while EBA autoantibodies recognize a 290 kDa collagenous glycoprotein (lamina densa)[13,34].

SALT-SPLIT SKIN IF STUDIES

Salt split skin refers to skin that has been artificially separated through the lamina lucida by incubation of normal or patient skin in 1 mol/L NaCl for 72 hours at 4°C. The technique was first developed in 1984 by Gammon et al[34], in which normal human skin was used as a substrate and patient serum as test (indirect salt-split skin technique). It has been shown that split skin is more sensitive than intact skin for detecting circulating auto-antibodies[43]. Pemphigoid antibodies bind solely to the lower

aspect of the basal keratinocytes (the blister roof) in 80% of cases; in about 20% of cases, the antibodies bind to both the lower basal keratinocytes (the roof), and the superior aspect of the base (the blister floor). Rarely, it has been reported that pemphigoid antibodies may bind solely to the floor[10,19]. In these cases toad skin substrate may be used to confirm the diagnosis as they contain only BP antigens and not those of EBA[19]

Serum may be negative for auto-antibodies in upto 50% of cases of BP. Hence Kowalewski et al[5] used DIF on split skin obtained from the patient (direct salt-split skin technique[10,13]). When this technique is used in pemphigoid, IgG is present on the roof or on the roof and the floor. C3 will usually bind to both the roof and floor[19].

CLINICAL VARIANTS:

1. Drug associated bullous pemphigoid:

   Furosemide, phenacetin, and various penicillins have been associated with bullous pemphigoid. It is possible that drug hypersensitivity may not be a cause of pemphigoid, but that the patients had subclinical pemphigoid with a superimposed drug eruption that produces damage to the dermo-epidermal junction.

2. Pemphigoid localized to lower extremities:

   Histologically, these reveal a cell-poor pattern and have positive IF findings less frequently (50%) than routine bullous pemphigoid.

3. Vesicular pemphigoid:

   This variant is worthy of designation only because of its clinical similarity to dermatitis herpetiformis, which may lead to misdiagnosis.

CICATRICAL PEMPHIGOID:

Cicatrical pemphigoid is a bullous disorder characterized by a chronic course and predilection for mucosal surfaces with little skin involvement[10,16]. It was initially termed as benign mucosal pemphigoid by Lever in 1953[49] and was subsequently redesignated as CP. Most patients are elderly, and there is a male predilection. Oral blisters are present in virtually all cases, ocular involvement is observed in 75% and cutaneous involvement in 33%[10,49]. The oral lesions are usually small blisters that subsequently erode and ulcerate. Other mucosal surfaces, including the larynx, esophagus, nose, vulva, and anus, may also be involved. Scarring is less evident in these locations than in the conjunctiva, where erythema without blisters and ulceration and subsequent scarring are the rule. Unilateral blindness can occur in up to 20% of cases.

HISTOPATHOLOGY:

In cutaneous lesions, a subepidermal blister develops that may extend down adnexa. Neutrophils, histiocytes, and lymphocytes predominate in the inflammatory infiltrate. Lamellar fibrosis beneath the epidermis is a hallmark[10] but may not be present in the initial lesions. Mucosal lesions generally have a lichenoid lymphocytic infiltrate in which neutrophils or eosinophils or both may be present. The changes are often nonspecific in both mucosal and cutaneous lesions, but the above features should lead to consideration of cicatricial pemphigoid.

IMMUNOFLUORESCENCE:

DIF studies reveal linear BMZ IgG and C3 in lesional and perilesional skin in approximately 80% of cases. Biopsy specimens from ocular lesions frequently give negative results when compared to those from the oral mucosa[14]. Deposition of immunoreactants along the basement membrane zone of mucous glands appears to be a specific finding[19]. Patients with cicatricial conjunctivitis that display only linear IgA deposits and are best considered a scarring variant of linear IgA dermatosis. Compared to bullous pemhigoid, the presence of circulating auto-antibodies against the BMZ is less frequent[23].

It is noteworthy that autoantibodies from patients with CP tend to target the C-terminus of BPAg2, whereas those from patients with BP, pemphigoid gestationis, and lamina-lucida type linear IgA bullous dermatosis typically target the NC16A domain of BPAg2. Some authors[24,49] have considered CP to be an abortive, less severe form of bullous pemphigoid although this has not been universally accepted[57].

HERPES GESTATIONIS:

Herpes or pemphigoid gestationis is a blistering disorder that usually develops during the second to third trimester in pregnant women. It is estimated to occur in 1 in 50,000 pregnancies . Intensely pruritic, urticarial lesions usually develop on the abdomen with subsequent involvement of the extremities, hands, and feet. They usually progress to tense vesicles and bullae, some herpetiform. The lesions can persist for more than 3 wks post-partum[17].The disorder may recur with subsequent pregnancies, menstrual periods, or the use of birth control pills. The course for the mother is benign. There was a debate concerning the possibility of an increased incidence and risk of fetal morbidity and mortality. However, data obtained in the 1990s indicates that no such risk can be confirmed. The infant, however, may be born with a mild, transient, vesiculobullous eruption secondary to transplacental transfer of the mediating antibody. Studies[17] have shown a high frequency of HLA-B8 and association with autoimmune thyroiditis in these patients.

HISTOPATHOLOGY:

In zones of erythema and edema, there is a perivascular infiltrate composed of lymphocytes and eosinophils. There is marked papillary dermal edema. There may be spongiosis, which may be eosinophilic in type. Focal necrosis of the basal keratinocytes, which has been emphasized by some authors, leads to a subepidermal blister.

IMMUNOFLUORESCENCE:

DIF testing reveals linear deposition of C3 in perilesional skin in virtually all patients. These may also be found in the newborn infant[14]. IgG is similarly deposited in 30% to 40%. The BPAg2 is the principal antigen, but the BPAg1 is recognized in some patients as well. Circulating IgG1 class anti-BMZ antibodies, known as the 'pemphigus gestationis factor', are usually not detectable in this disease but some sera do bind C3 to the BMZ[14]. Recent studies have identified serum PG factor in over 90% of the cases using complement-binding IIF technique[19].

SUB-EPIDERMAL BULLOUS DERMATOSES WITH AUTOIMMUNITY TO TYPE VII COLLAGEN

EPIDERMOLYSIS BULLOSA ACQUISITA:

Classically, EBA is a rare, non-inherited disorder of acquired skin fragility arising beyond the childhood period[45]. Traumatic blisters developing on inflammatory bases with a predilection for acral areas are seen which may mimic BP or Dermatitis herpetiformis. The classic non-inflammatory mechano-bullous pattern may follow or may be the initial manifestation[19]. Scarring and milia formation ensue. A characteristic nail dystrophy and alopecia are noted. This presentation is associated with malignant lymphoma, amyloidosis, and colitis or enteritis[40,45]. Some patients with EBA may have significant involvement of oral and conjunctival mucosa and therefore may be cicatricial pemphigoid-

like. However, acral lesions are prominent and nail dystrophy is noted.

HISTOPATHOLOGY:

The bullous pemphigoid-like presentation described above is the most common form of EBA. The subepidermal blisters are inflammatory. The predominant infiltrating cells are lymphocytes and neutrophils in perivascular and focal interstitial array. Eosinophils are present in variable numbers. In the classic form, the subepidermal blisters are noninflammatory, and fibrosis and milia formation are often present.

IMMUNOFLUORESCENCE:

Examination of perilesional skin using DIF reveals linear deposition of complement at the basement membrane zone in the vast majority of cases. IgG is by far the most common immunoglobulin found, but IgM and IgA may be present as well. Increasing numbers of immunoglobulin subclasses noted at the dermo-epidermal junction favor a diagnosis of EBA over bullous pemphigoid. The presence of linear C3 at the dermo-epidermal junction alone favors bullous pemphigoid over EBA. However, use of routine DIF cannot reliably distinguish between bullous pemphigoid and EBA.

Salt split skin techniques with IIF can reliably differentiate EBA from BP. However upto 50% of patients with EBA may lack circulating auto-antibodies[13]. Hence, split skin methods employing perilesional normal skin (DIF) from the patient can be used.

The antibodies in EBA have specificity for a 290 kDa protein (type VII collagen) which is the main component of the anchoring fibrils[25]. The antibodies are thus deposited beneath the lamina densa. Therefore, on salt-split skin studies, IgG is on the floor and not on the roof of the split.

BULLOUS SYSTEMIC LUPUS ERYTHEMATOSUS:

Vesicles and bullae may develop in patients with systemic lupus erythematosus which is a rare cutaneous mannifestation. The disease may clinically mimic bullous pemphigoid or dermatitis herpetiformis[4]. However, in contrast to dermatitis herpetiformis, the lesions are usually widespread, nonpruritic and are neither symmetrical nor do they have a predilection for extensor surfaces of arms, elbows, or scalp. The lesions may be photodistributed. These patients can rarely have classic lesions of discoid, systemic or subacute cutaneous lupus erythematosus when they develop blisters. Bullous lupus erythematosus is most common in women, particularly black women. No correlation with clinical activity of lupus erythematosus was apparent [10] although this view is contested by some authors[4]. Some patients with EBA can progress to systemic lupus erythematosus (SLE).

HISTOPATHOLOGY:

Three histologic patterns have been identified in such lesions. The first is striking basal layer vacuolization with subsequent blister formation. The second is vasculitis with subepidermal blister and pustule formation. The third and most common is a dermatitis herpetiformis-like histologic pattern[10,19]. Approximately 25% of cases are said to have a small-vessel, neutrophil-rich leukocytoclastic vasculitis beneath the blister. Another histologic finding that is not emphasized in most case reports is the presence of dermal mucin and hyaluronic acid as defined by Alcian blue stain at pH 2.5[10].

IMMUNOFLUORESCENCE:

In all reported cases, IgG and C3 are deposited at the epidermal basement membrane zone. The pattern is ‘granular bandlike’ in more than 60% cases, linear in approximately 40% or rarely fibrillar[19]. IgM and IgA are present in approximately 50% and 60% of cases, respectively. The target antigen is type VII collagen and immunofluorescnece on salt-split skin shows linear deposits on the dermal side,

as seen in EBA[5]. In general, granular patterns represent deposition of circulating immune complexes in situ or in situ binding of antigen and antibody in non-compartmentalized zones. Therefore, perhaps some of the cases represent tubular or linear deposition obscured by confluent granular bands (positive lupus band test). A salt-split skin preparation using patient serum reveals localization to the split floor as in EBA.

Bullous SLE may be divided into types I and II on the basis of the presence or absence of antibodies to type VII collagen. Failure to demonstrate these auto-antibodies with both IIF and direct immunoelectron microscopy permits classification as type II. The significance of this classification is unclear as clinical differences between these two types are not apparent[19].

SUBEPIDERMAL IgA MEDIATED VESICOBULLOUS DISORDERS

DERMATITIS HERPETIFORMIS

This is an intensely pruritic, chronic recurrent dermatitis that has a slight male predilection. The lesions usually develop in young to middle-aged adults as intensely pruritic, symmetrical grouped papulovesicles, vesicles, or crusts on erythematous bases. Oral lesions are absent. The elbows, knees, buttocks, scapula, and scalp are commonly involved. There is a high incidence (about 90%) of gluten-sensitive enteropathy, and an increased but rare risk of lymphoma. Over 80% of these patients have HLA-B8, a similar incidence to that in celiac disease[32,48].

HISTOPATHOLOGY:

The typical histologic features are best observed in erythematous skin adjacent to early blisters. In these zones, neutrophils accumulate at the tips of dermal papillae. With an increase in size to microabscesses, a significant admixture of eosinophils may be noted. As microabscesses form, a

separation develops between the tips of the dermal papillae and the overlying epidermis, so that early blisters are multiloculated. Within 1 to 2 days, the rete ridges lose their attachment to the dermis, and the blisters then become unilocular. At this time, the characteristic papillary microabscesses may be observed at the blister periphery. For this reason, the inclusion of perivesicular skin in the biopsy specimen is of utmost value[10]. Apoptotic keratinocytes may be noted above the papillary microabscesses.

IMMUNOFLUORESCENCE:

Granular IgA deposits are found alone or in combination with other immunoreactants within the dermal papillae in over 95% of cases. Fibrillary IgA deposits may also be present. Normal buttock skin is the recommended site of biopsy[14]. The IgA autoantibodies are dimeric, polyclonal and belong to the IgA1 class[19,53]. The presence of IgA within the skin is not altered by dapsone therapy[10]. After as long as 2 years, a gluten-free diet results in the disappearance of IgA from the skin. Negative results of DIF testing of two appropriately selected biopsy sites are a strong indication that the patient does not have dermatitis herpetiformis.

Serum anti-endomysial[19] and anti-transglutaminase antibodies are both specific and sensitive in the diagnosis of dermatitis herpetiformis. These occur in 70-80% of patients with DH and in a somewhat lower percentage of patients with only celiac disease[14]. Anti-gliadin antibodies can also be found.

LINEAR IGA DERMATOSIS:

A group of bullous disorders mediated by IgA antibodies with differing specificities for epidermal basement membrane zone antigens has been labeled linear IgA dermatosis (LAD). There are two relatively definitive clinical phenotypes that are based on patient age and clinical features - adult linear IgA dermatosis and childhood linear IgA dermatosis (chronic benign bullous dermatosis of childhood). They differ slightly in their clinical presentations but have identical immunopathological

features. Third, a clinical phenotype similar to cicatricial pemphigoid has been described. Another subset of patients has been described with drug-induced linear IgA dermatosis. On the basis of immunoelectromicroscopic localization of the IgA deposition, there are at least two distinct types of LAD: a lamina lucida type and a sublamina densa type. Some of the sublamina densa types of LAD are best classified as IgA epidermolysis bullosa acquisita. The IgA deposits lack J chains and are of the IgA1 class[19,32]. There is a reported increase in the frequency of associated lymphoproliferative disorders in these patients[19].

ADULT TYPE:

Vesicles and bullae develop in patients usually over 40 years of age, with a slight female predilection. The lesions are less symmetrical and less pruritic than those in dermatitis herpetiformis but are distributed in similar locations. Ocular and oral lesions may be present in up to 50% of cases. Plantar and palmar bullae may develop in contrast to dermatitis herpetiformis. Cutaneous lesions in LAD are heterogeneous and may mimic other bullous diseases. Linear IgA dermatosis has been associated with an increased risk of lymphoma. Ulcerative colitis has been correlated with LAD in various studies. A rare association with SLE has been reported.

HISTOPATHOLOGY:

The features are similar, if not identical, to dermatitis herpetiformis[10,19]. According to some, there is lesser tendency for papillary microabscess formation and greater tendency for uniform neutrophil infiltration along the entire dermo-epidermal junction and rete in inflamed skin.

IMMUNOFLUORESCENCE:

As this test defines the disease, DIF reveals linear IgA along the basement membrane zone in perilesional skin in 100% of cases. In the lamina lucida type of LAD, IgA antibodies bind to the epidermal side of salt-split skin, whereas in the sublamina densa type, such as IgA-mediated epidermolysis bullosa acquisita, IgA antibodies bind to the dermal side of salt-split skin. Studies have a shown a regional variation in the deposition of antibodies in these patients with volar aspect of

forearm being negative in a large number of cases[6,19].

DRUG-INDUCED LINEAR IgA DERMATOSES:

It is important to note that drug therapy has frequently been associated with adult-type linear IgA dermatosis[10]. Vancomycin, lithium, diclofenac, captopril, cefamandole, and somatostatin have been associated with such presentations. Histologically, the changes are identical to idiopathic linear IgA dermatosis in most cases. In some cases, there is an associated lymphoeosinophilic infiltrate in combination with the interface neutrophilic infiltration.

CHILDHOOD TYPE:

Originally known as chronic bullous dermatosis of childhood, this disorder presents in prepubertal, often preschool children, and rarely in infancy. Vesicles or bullae develop on an erythematous or normal base, occasionally giving rise to a so-called 'string of pearls' or 'cluster of jewels'[19], a characteristic lesion in which peripheral vesicles develop on a polycyclic plaque. They involve the buttocks, lower abdomen, and genitalia, and characteristically have a perioral distribution on the face. Oral lesions may occur. The disorder usually remits by age 6 to 8.

HISTOPATHOLOGY:

The features are similar to those of the adult-type disease. Some cases, however, resemble bullous pemphigoid because of the presence of eosinophils.

IMMUNOFLUORESCENCE:

DIF testing reveals linearly deposited IgA in virtually 100% of cases. At this time, the targeted antigens are thought to be identical to those noted in the adult-type disease.

OTHER BULLOUS DISEASES WITH IMMUNOCYTOCHEMICAL AND IMMUNOPATHOLOGICAL FINDINGS IN THE SKIN AND SERA[14]

Certain other bullous dermatoses, notably hereditary forms of epidermolysis bullosa, lichen planus pemphigoides, porphyria and erythema multiforme have features that are recognizable by

immunopathological studies. While such immunologic features are of some diagnostic value, they do not have a high degree of disease specifity.

RESULTS AND ANALYSIS

1. DETAILS OF SKIN BIOPSY SAMPLES:

Total no of biopsies received during the study period	8271
Total number of punch biopsies from skin	1244
Total number of immunobullous lesions during study period	82

Of the 82 cases, we included only those cases for which both direct immunofluorescence and histopathology had been done (total 40 cases). The remaining 42 cases had either only histopathology or immunofluorescence done and hence were not included.

2. AGE DISTRIBUTION OF IMMUNOBULLOUS DISORDERS:

Of the 19 cases of pemphigus vulgaris, the majority of the cases i.e. 7/19 cases (36.8%) were within the ages of 21 and 30 (mean age 34 yrs). A total of 14/19 cases (73.6%) were between 21 and 50 yrs of age. The youngest patient in the entire study was 3 yrs old.

10/13 cases with bullous pemphigoid were between the ages of 51 and 80 (mean age 60 yrs). Of these, 6/10 cases (60%) were between the ages of 51 and 60. The oldest patient in the study belonged to this group and was 91 yrs old.

Pemphigus foliaceous (mean age 50 yrs), pemphigus vegetans and bullous SLE were seen between the ages of 31 and 70. The two patients with chronic bullous dermatoses of childhood (CBDC) were 6 and 7 yrs old.

TABLE-1: AGE DISTRIBUTION OF IMMUNOBULLOUS LESIONS

S NO	DISORDER	AGE IN YEARS									
		1-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100
1	PEMPHIGUS VULGARIS	1	1	7	3	4	3	0	0	0	0
2	BULLOUS PEMPHIGOID	0	0	1	0	1	6	1	3	0	1
3	PEMPHIGUS FOLIACEOUS	0	0	1	0	0	2	1	0	0	0
4	PEMPHIGUS VEGETANS	0	0	0	1	0	0	0	0	0	0
5	CHRONIC BULLOUS DERMATOSES OF CHILDHOOD	2	0	0	0	0	0	0	0	0	0
6	BULLOUS SLE	0	0	0	0	1	0	0	0	0	0
	TOTAL	3	1	9	4	6	11	2	3	0	1

3. SEX DISTRIBUTION OF IMMUNOBULLOUS DISORDERS: TABLE-2

S NO	DISORDER	NO OF CASES	SEX			
			MALE		FEMALE	
			NO.	%	NO.	%
1	PEMPHIGUS VULGARIS	19	7	36.8	12	63.2
2	BULLOUS PEMPHIGOID	13	2	15.3	11	84.6
3	PEMPHIGUS FOLIACEOUS	4	2	50	2	50
4	PEMPHIGUS VEGETANS	1	0	0	1	100
5	CHRONIC BULLOUS DERMATOSES OF CHILDHOOD	2	1	50	1	50
6	BULLOUS SLE	1	0	0	1	100
	TOTAL	4	12	30	28	70

In the entire study, the majority of the patients (28/40) were women (70%). The M:F ratio was 1: 2.3

This pattern was observed in all categories of diseases studied except for pemphigus foliaceous and chronic bullous dermatoses of childhood, where there was equal sex distribution.

4. DISTRIBUTION OF VARIOUS TYPES OF IMMUNOBULLOUS LESIONS: TABLE-3

S NO	DISORDER	NO OF CASES	PERCENTAGE
1	PEMPHIGUS VULGARIS	19	47.5

2	BULLOUS PEMPHIGOID	13	32.5
3	PEMPHIGUS FOLIACEOUS	4	10
4	PEMPHIGUS VEGETANS	1	2.5
5	CHRONIC BULLOUS DERMATOSES OF CHILDHOOD	2	5
6	BULLOUS SLE	1	2.5
	TOTAL	40	100

19/40 cases (47.5%) studied were of pemphigus vulgaris, while 13/40 cases (32.5%) were of bullous pemphigoid.

5. HISTOPATHOLOGICAL CHANGES IN VARIOUS IMMUNOBULLOUS LESIONS:

a) PEMPHIGUS VULGARIS

TABLE-4

S NO	HISTOPATHOLOGICAL CHANGES	NO OF CASES	PERCENTAGE
1	SPONGIOSIS	6	31.5
2	ACANTHOLYSIS	14	73.7
3	SUPRABASAL BLISTER	15	78.9
4	EXTENSION INTO FOLLICULAR EP	9	47.3
5	DERMAL INFLAMMATION		
	(i) MILD	8	42.1
	(ii) MODERATE	6	31.6
	(iii) MARKED	4	21.1
6	NON-SPECIFIC HISTOLOGY	4	21.1

A majority of the cases showed acantholysis and suprabasal cleavage with the

classical “tomb-stone” (fig.2) appearance (73.7% and 78.9% respectively).

Extension of acantholysis into the follicular epithelium (fig.3) was seen in almost half the cases studied (47.3%)

Two cases (10.5%) showed intact epidermis with a separated strip of degenerate squamous epithelium and acantholytic cells. Both cases showed only mild perivascular dermal inflammation with no evidence of spongiosis.

Two other cases (10.5%), both from the oral mucosa, showed non-specific histological findings. One of these cases had a dense band-like lymphohistiocytic infiltrate within the lamina propria (fig. 4), consistent with oral lichen planus. The other case showed ulceration, dense mixed inflammation of the lamina propria (fig.5) and focal lymphoid aggregates within a minor salivary gland lobule, suggestive but not diagnostic of chelitis glandularis. Both cases, in addition to the above said features, showed spongiosis.

b) BULLOUS PEMPHIGOID:

TABLE-5

SNO	HISTOPATHOLOGICAL CHANGES	NO OF CASES	PERCENTAGE

1	SUB-EPIDERMAL BLISTER	11	84.6
2	SUPERFICIAL DERMAL EDEMA	7	53.8
3	DERMAL INFLAMMATION		
	(i) CELL RICH	11	84.6
	(ii) CELL POOR	2	15.4
4	PREDOMINANT INFLAMMATORY CELL		
	(i) EOSINOPHILS	8	61.5
	(ii) MONONUCLEAR	5	38.5
5	SPONGIOSIS	1	7.7

11/13 cases (84.6%) showed a sub-epidermal blister (fig8). Of the two remaining cases, one case showed an intact epidermis with spongiosis. The superficial dermis showed edema and an eosinophil rich perivascular inflammatory infiltrate (consistent with early bullous pemphigoid) (fig.11). This was the only case with spongiosis (7.7%)

The other case showed only dermis with superficial mild perivascular lymphocytic infiltrate.

2/13 cases (15.4%) showed a cell-poor pattern (fig.10) and 8/13 cases (61.5%) showed the predominant inflammatory cell to be eosinophils (fig. 9).

c) PEMPHIGUS FOLIACEOUS:

TABLE-6

S NO	HISTOPATHOLOGICAL CHANGES	NO OF CASES	PERCENTAGE
1	SPONGIOSIS	4	100
2	SUB-CORNEAL BLISTER	3	75
3	ACANTHOLYTIC/ DYSKERATOTIC KERATINOCYTES	3	75
4	DERMAL INFLAMMATION	4	100

3 /4 cases (75%) showed a sub-corneal blister (fig.14) with occasional acantholytic

keratinocytes (fig. 15). One case showed epidermal spongiosis with absent granular layer and stratum corneum with neutrophilic collections (fig.16). The dermis showed a perivascular lymphocytic infiltrate. A separated fragment of lamellated keratin with neutrophils was also seen. There was no evidence of acantholysis and hence a diagnosis of pustular psoriasis was suggested, which was the clinical differential diagnosis.

d) PEMPHIGUS VEGETANS:

Histopathology showed epidermis with a supra-basal blister, acantholysis, marked acanthosis and papillomatosis (fig. 18). Focal spongiosis was also seen. The blister cavity showed acantholytic cells and neutrophils. Acantholysis also involved the follicular epithelium.

e) CHRONIC BULLOUS DERMATOSES OF CHILDHOOD:

One case showed epidermis with spongiosis and a sub-epidermal bulla containing neutrophils, eosinophils and scattered lymphocytes (fig 21, 22). The superficial dermis shows edema and a dense inflammatory cell infiltrate.

The other case showed only a sub-epidermal cleft filled with neutrophils and plasma. Superficial dermal mild perivascular inflammation composed predominantly of neutrophils was also seen.

f) BULLOUS SLE:

This case showed epidermis with a sub-epidermal cleft containing neutrophils and plasma (fig. 24). The superficial dermis showed a mild perivascular lymphocytic infiltrate with evidence of vasculitis (fig. 25). Special stains (Alcian Blue) revealed mild increase in dermal mucin.

6) DIRECT IMMUNOFLUORESCENCE FINDINGS:
TABLE-7

SORDER	NO OF CASES	DIF POSITIVE CASES		PATTERN	INTENSITY OF STAINING			ANTIBODIES DETECTED					
		NO	%		STRONG	MOD STRONG	WEAK	IgG	IgA	(+) IgM	(+) IgA	IgG+C3	IgG-
PV	19	18	94.7	ICS	10	7	1	3	0	2	1	11	
BP	13	12	92.3	Linear BMZ	5	6	1	0	0	1	1	8	
PF	4	4	100	ICS *	2	2	0	2	0	1	0	0	
VEG	1	1	100	ICS	0	1	0	0	0	0	0	1	
CBDC	2	2	100	Linear BMZ	0	2	0	0	1	1	1	0	
ULLOUS SLE	1	1	100	Granular BMZ	0	1	0	0	0	0	0	1	

* 3/ 4 cases had ICS staining restricted to upper third of epidermis
one case had full thickness ICS staining

a) PEMPHIGUS VULGARIS:

5/ 19 cases (26.3%) showed (InterCellular Substance) ICS staining pattern restricted to the lower third of the epidermis while the rest of the 13 cases (68.4%), which had shown a positive reaction, showed full thickness ICS pattern of staining (fig. 6,7).

7/ 19 cases (36.8%) showed a weaker ICS staining pattern for C3 when compared to IgG. One case had shown staining with IgM in addition to IgG and C3 while another case showed a positive reaction with both IgM and IgA along with IgG and Fibrinogen. 1/ 19 cases was negative for DIF.

b) BULLOUS PEMPHIGOID:

Of the 12 case showing a positive reaction (92.3%), 8 cases (61.5%) showed a linear Basement Membrane Zone (BMZ) pattern of staining with IgG (fig. 12) and C3 (fig. 13) alone (with varying intensity between IgG and C3).

Positive reaction with IgM was seen in one case, while another showed positivity with IgA and a third case showed positivity with Fibrinogen, all three of which were in addition to IgG and C3. 1/ 13 cases was negative for DIF.

c) PEMPHIGUS FOLIACEOUS:

3/ 4 cases (75%) showed ICS pattern of staining restricted to the upper third of the epidermis (fig. 17) while one case showed full thickness ICS staining pattern. IgM was positive in one case in addition to IgG and C3.

d) PEMPHIGUS VEGETANS:

This case showed a moderately strong full thickness ICS staining pattern with

IgG (fig. 23) and a weak ICS staining pattern with C3.

e) CHRONIC BULLOUS DERMATOSES OF CHILDHOOD:

One case showed a weak positive linear BMZ pattern of staining with IgM and IgG in addition to a moderately positive staining pattern with IgA.

The other case showed a moderately strong linear BMZ pattern of staining with IgA alone (fig. 23).

f) BULLOUS SLE:

A moderately strong granular BMZ pattern of staining (fig.26, 27) was seen with IgG and C3 in this patient.

7. CORRELATION OF DIF AND HISTOPATHOLOGY FINDINGS:

TABLE-8

S NO	DISORDER	NO OF CASES	CASES WITH POSITIVE DIF	CASES WITH POSITIVE HPE AND DIF	CASES WITH POSITIVE DIF AND DISCORDANT HPE	CASES WITH NEGATIVE DIF AND DIAGNOSTIC HPE
1	PV	19	18 (94.7%)	14 (73.6%)	4 (21.1%)	1 (5.3%)
2	BP	13	12 (92.3%)	11 (84.6%)	1 (7.7%)	1 (7.7%)
3	PF	4	4 (100.0%)	3 (75%)	1(25%)	0 (0)
4	CBDC	2	2 (100.0%)	2 (100%)	0 (0)	0 (0)
5	VEG	1	1 (100.0%)	1 (100%)	0 (0)	0 (0)
6	BULLOUS SLE	1	1 (100.0%)	1 (100%)	0 (0)	0 (0)

PEMPHIGUS VULGARIS:

18/19 cases (94.7%) were positive for DIF showing an ICS pattern of staining (fig. 6) within the epidermis.

The patient with a negative DIF was a 26 yr old female who had presented with multiple flaccid blisters all over the body with involvement of the oral mucosa for a period of three months. Histopathology showed features characteristic of pemphigus vulgaris.

Of the 18 cases that were positive with DIF, four cases showed discordant findings with histopathology. Two of these four cases did not show a blister. Both showed an intact epidermis with a separated strip of degenerate epithelium.

The other two cases were from the oral mucosa. One of them showed a band-like dense infiltrate of lymphohistiocytes in the lamina propria with the surface epithelium showing spongiosis (fig. 4) and no evidence of blister formation. The findings were consistent with oral lichen planus. DIF showed focal weak ICS pattern of staining with IgG alone.

The other case showed epithelial ulceration and spongiosis with sub-epithelial dense mixed inflammation (fig. 5). A salivary gland lobule with a focal lymphoid aggregate was also seen. There was no acantholysis or bulla on histopathology. The findings were suggestive but not diagnostic of chelitis glandularis. DIF showed strong positive ICS pattern of staining with IgG, IgM and Fib along with weak ICS staining with IgA.

BULLOUS PEMPHIGOID:

12/ 13 cases (92.3%) showed a positive linear BMZ pattern of staining on DIF. The

case which showed a negative reaction was a 28 yr old female with tense bullae on both hands and legs. Histopathology showed features characteristic of bullous pemphigoid.

One of the twelve cases which showed a positive reaction, on histopathology showed only dermis with perivascular lymphocytic infiltrate. The epidermis was not seen.

PEMPHIGUS FOLIACEOUS:

3 / 4 cases (75%) showed ICS pattern of staining restricted to the upper 2/3rd of the epidermis on DIF (fig.17). The other case showed full thickness ICS pattern of staining but had a sub-corneal blister on histopathology with presence of acantholytic cells.

One of the three cases with the characteristic DIF pattern, on biopsy showed epidermal collections of neutrophils with absent granular layer (fig16). The diagnosis on histopathology was pustular psoriasis. There was no evidence of spongiosis or acantholysis.

8. DETAILS OF DISCORDANT CASES:

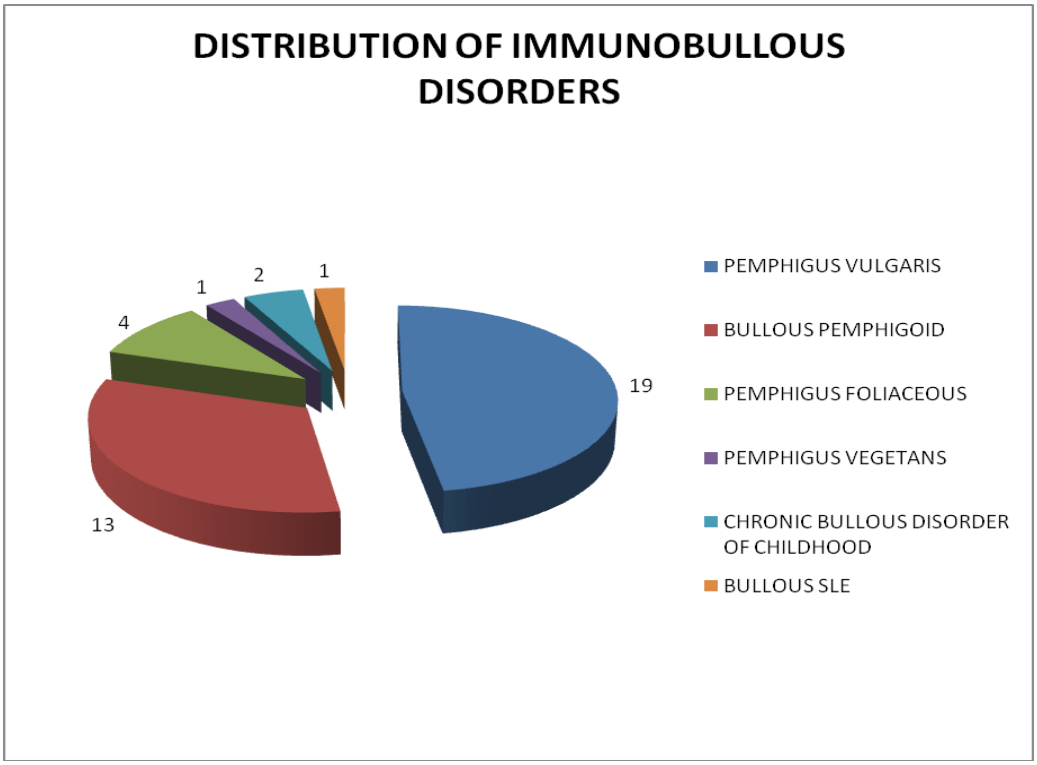
TABLE-9

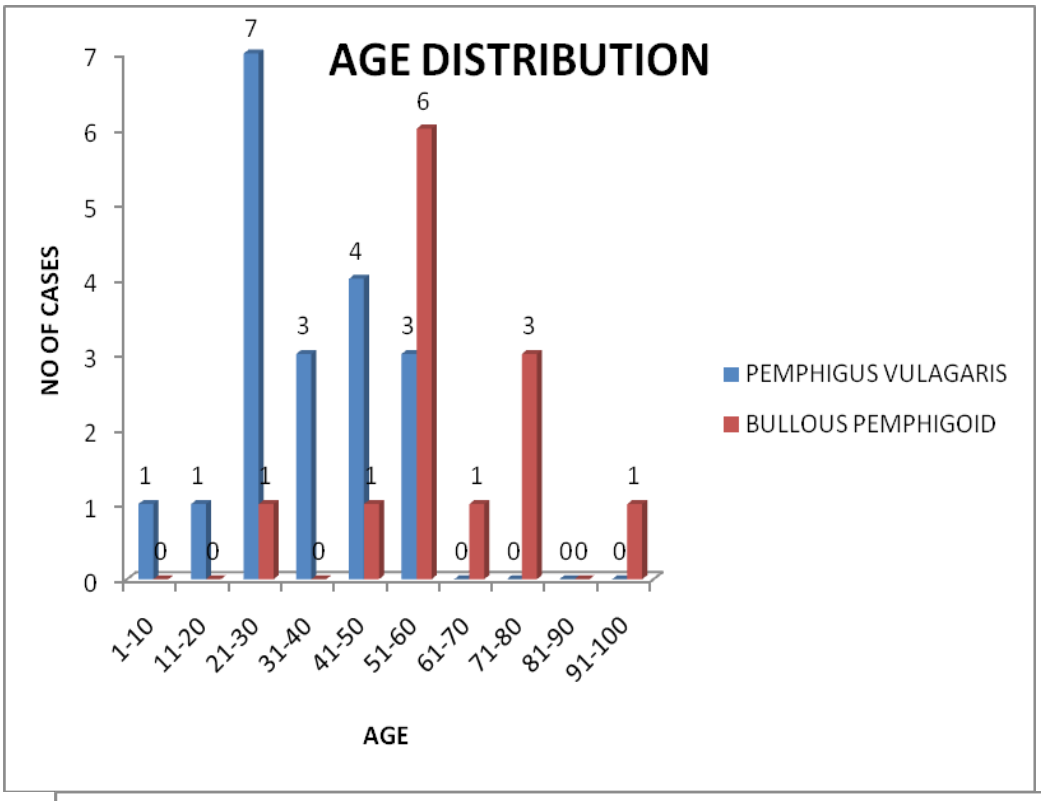
S NO	CLINICAL DIFFERENTIAL DIAGNOSIS	HPE DIAGNOSIS	DIF FINDINGS	ANTIBODY	FOLLOW-UP
1	Pemphigus vulgaris	Chelitis Glandularis	Strong ICS	IgG, IgM, Fib	Was started on treatment for PV, was lost on follow-up
2	Pemphigus vulagris, Oral Lichen planus	Oral Lichen Planus	Weak ICS	IgG only	Was started on treatment for PV, was lost on follow-up

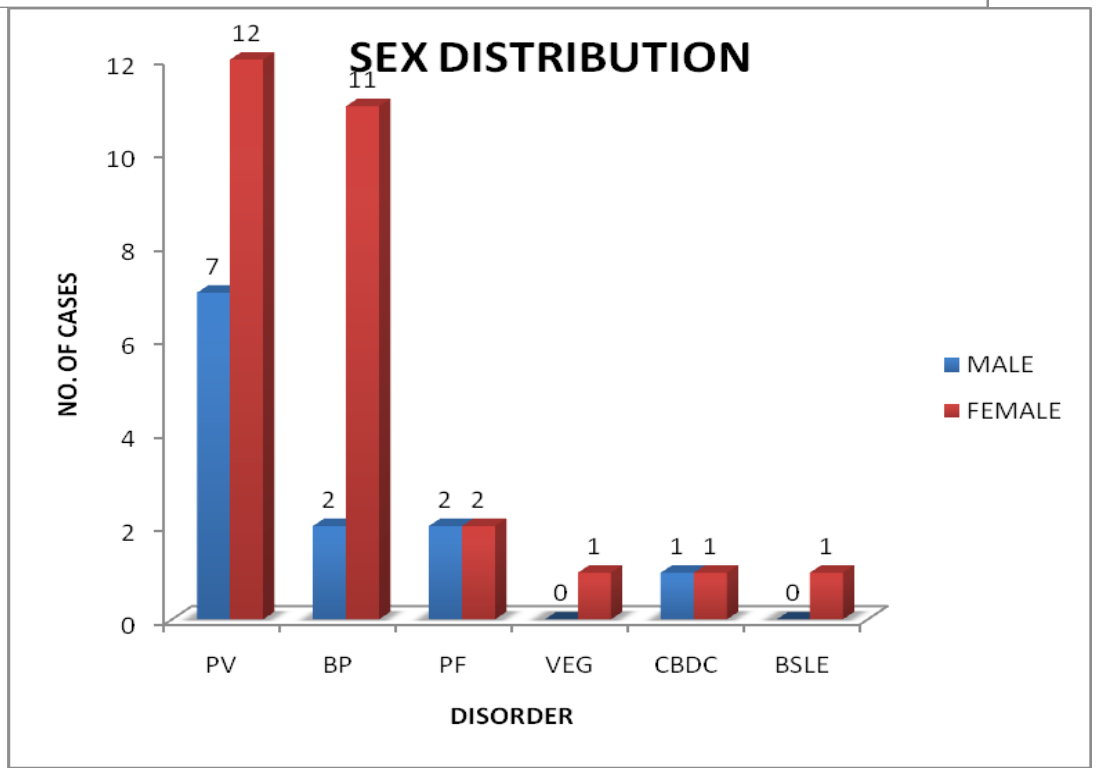
3	Pemphigus vulagris, Pemphigus foliaceous	Non-specific histology *	Strong ICS	IgG - lower epidermis	responded to oral steroids. Repeat bx not done
4	Pemphigus vulgaris	Non- specific histology *	Moderately strong ICS	IgG only	responded to oral steroids. On 5th pulse. No new lesions.
5	Pemphigus vulgaris	Pemphigus vulgaris	Negative	-	Was started on treatment for PV, lost on follow-up
6	Dermatitis herpetiformis, Bullous pemphigoid	Bullous pemphigoid	Negative	-	Was started on treatment for BP, lost on follow-up
7	Bullous pemphigoid	Non-specific histology (no epidermis)	Linear BMZ	IgG, C3	Was started on treatment for BP, lost on follow-up
8	Pemphigus foliaceous, Pustular psoriasis	Pustular psoriasis	Moderately strong ICS - upper epidermis	IgG, C3	Treated for pemphigus foliaceous **

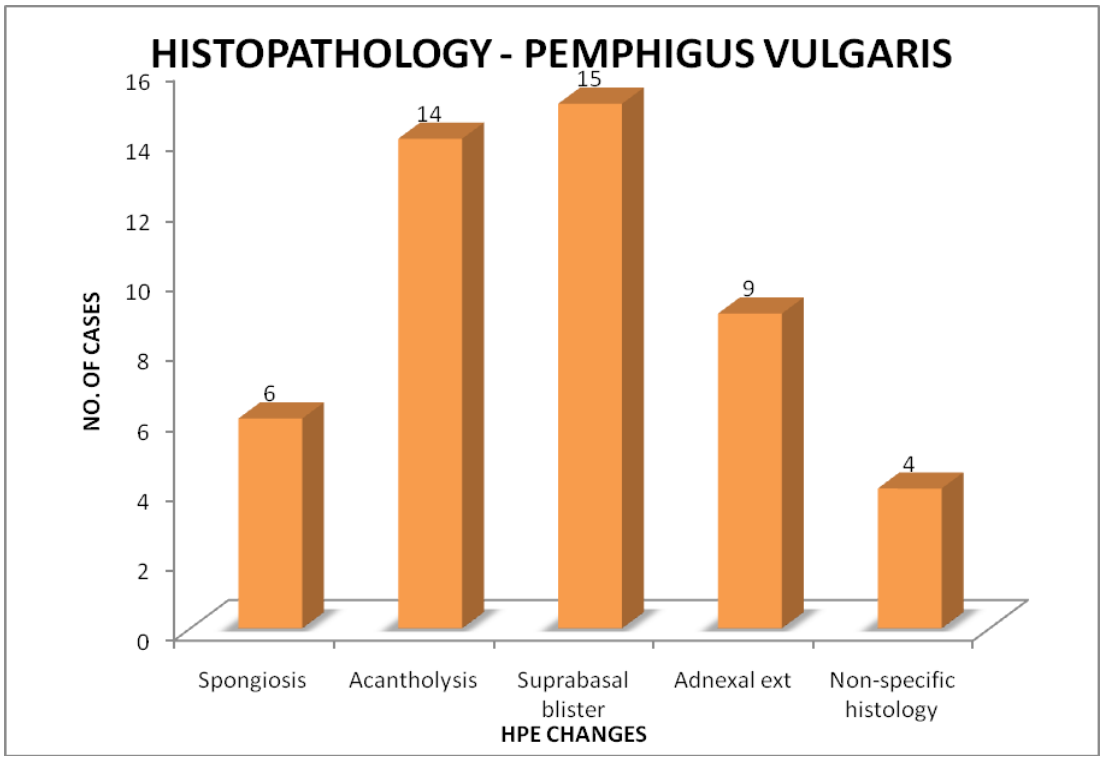
* both these cases showed separately lying strip of degenerate epithelium with occasional acantholytic cells

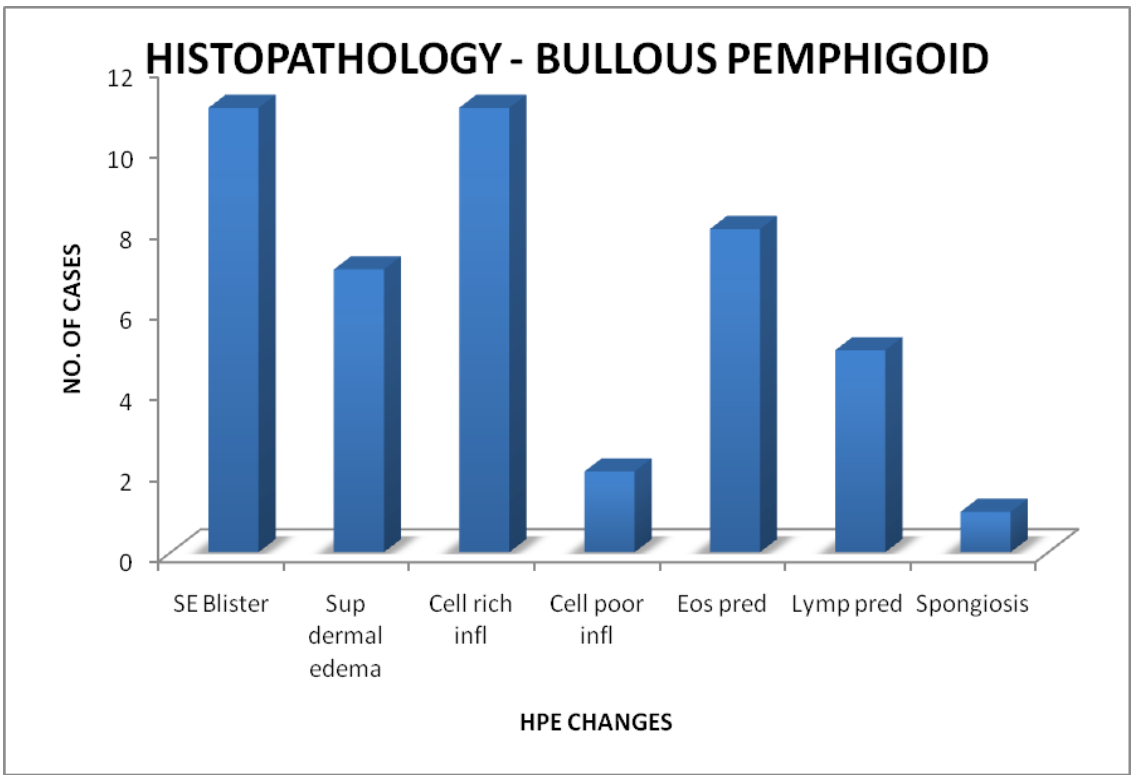
** This patient had presented with generalized pustular eruptions of skin. Since Hpe showed a sub-corneal pustular lesion suggestive of pustular psoriasis and DIF showed findings in favour pemphigus foliaceous, the patient was started on both methotrexate and steroids. A sample was sent to London for immunoblotting and ELISA which was positive for Desmoglein 1. On follow-up visit the patient was tapered off Mtx and is now on pulse therapy with corticosteroids. The patient has no new lesions. Hence a repeat biopsy was not done.

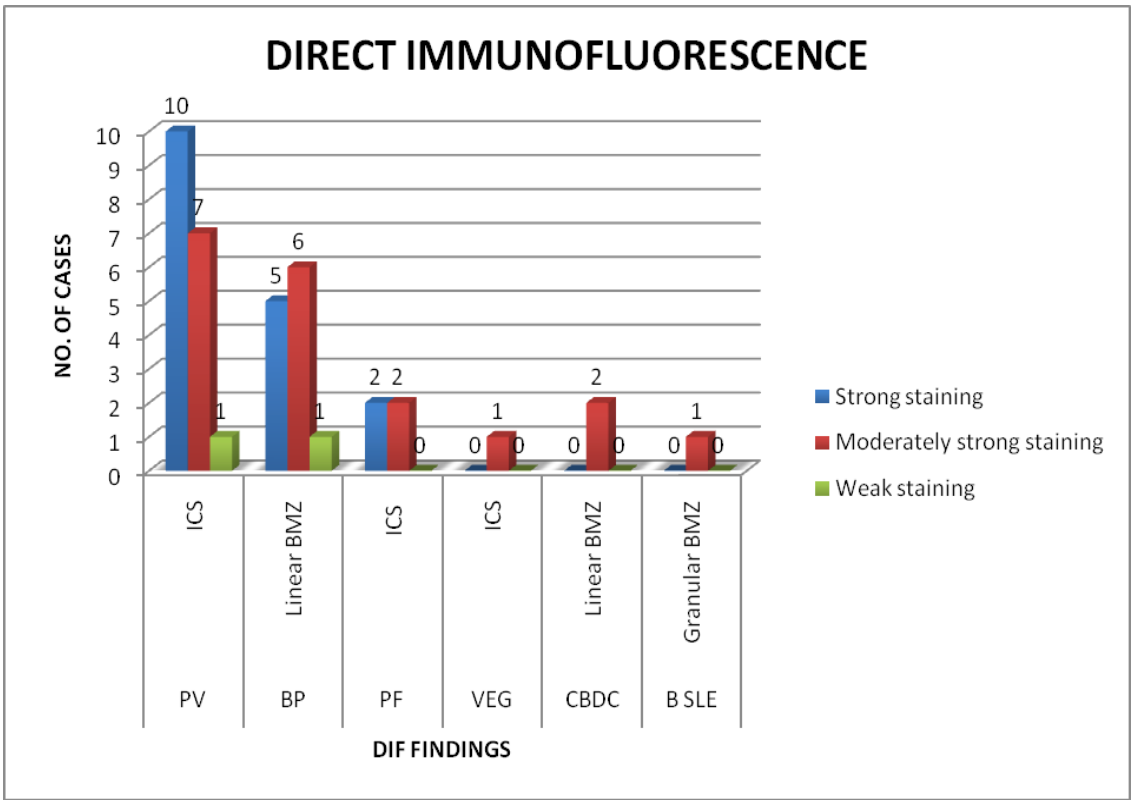

DISTRIBUTION OF IMMUNOBULLOUS DISORDERS
1
2
1
4
19
13
PEMPHIGUS VULGARIS
BULLOUS PEMPHIGOID
PEMPHIGUS FOLIACEOUS
PEMPHIGUS VEGETANS
CHRONIC BULLOUS DISORDER OF CHILDHOOD
BULLOUS SLE


AGE DISTRIBUTION
NO OF CASES
AGE
1-10
11-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
91-100
PEMPHIGUS VULAGARIS
BULLOUS PEMPHIGOID


SEX DISTRIBUTION
NO. OF CASES
DISORDER
PV
BP
PF
VEG
CBDC
BSLE
MALE
FEMALE


HISTOPATHOLOGY - PEMPHIGUS VULGARIS
NO. OF CASES
16
14
12
10
8
6
4
2
0
6
14
15
9
4
Spongiosis
Acantholysis
Suprabasal blister
Adnexal ext
Non-specific histology
HPE CHANGES


HISTOPATHOLOGY - BULLOUS PEMPHIGOID
NO. OF CASES
12
10
8
6
4
2
0
SE Blister
Sup dermal edema
Cell rich infl
Cell poor infl
Eos pred
Lymp pred
Spongiosis
HPE CHANGES


DIRECT IMMUNOFLUORESCENCE
NO. OF CASES
10
9
8
7
6
5
4
3
2
1
0
10
7
1
5
6
1
2 2
0
0
1
0
0
2
0
0
1
0
ICS
Linear BMZ
ICS
ICS
Linear BMZ
Granular BMZ
PV
BP
PF
VEG
CBDC
B SLE
DIF FINDINGS
Strong staining
Moderately strong staining
Weak staining

DISCUSSION

The immunobullous diseases are a group of autoimmune diseases in which components of the epidermis and basement membrane zone are the focus of attack, resulting in the formation of cutaneous and mucosal blisters. Early diagnosis and treatment of these severe and potentially life threatening disorders are vital.

Several disorders fall under the category of immunobullous diseases and are broadly classified as intra-epidermal and sub-epidermal blistering disorders. Intra-epidermal blistering disorders, apart from those mediated by immunological mechanisms, include inherited disorders like Darier's and Hailey-Hailey disease. Secondary damage by severe intercellular edema can also cause acantholysis, as seen in spongiotic dermatitis and transient acantholytic dermatosis. In a similar fashion, sub-epidermal blistering disorders include mechanobullous disorders like epidermolysis bullosa congenita, in addition to the immunobullous lesions.

Direct immunoflourescence (DIF) has been widely used as a supplement to clinical examination and histopathology in the evaluation of vesiculobullous lesions of the skin. As a prognostic tool it is limited by the fact that DIF continues to remain positive many years after the patient has gone into clinical remission.

PEMPHIGUS VULGARIS

The predominant lesion of the forty cases involved in the study was pemphigus

vulgaris (Table-3) with 19 cases (47.5%). This is in concordance to a few studies done earlier in India[2, 20]. DIF was positive in the majority of cases (18/19 cases, 94.7 %), similar to the study conducted by Krasey et al[30]. The mean age of the patients was 34 years which is consistent with that of Western literature[10,41] and studies conducted in India[2].

Though previous studies show no sex predilection[47], our study showed a predominance of females with 12/19 cases (63.2%) being women (Table-2). Similar results were observed in a study conducted in Libya[50].

Histopathology (Table-5) showed 15/19 cases (78.9%) with a suprabasal blister (fig.1, 2) and 9/19 cases (47.3%) showed acantholysis extending into follicular epithelium (fig.3).

DIF (Table-8) showed strongly positive intercellular pattern of staining (ICS) in 10/19 cases (fig.6, 7). Although rare cases with linear BMZ pattern have been described by Judd, Lever[30] and Beutner et al[30], we did not observe such a pattern in our study.

Most of the cases showed positive reactions with both IgG and C3. IgM and IgA were positive in a few cases along with IgG. Isolated IgA and IgM can be positive in pemphigus vulgaris as demonstrated by Judd and Lever[30] which was not seen in our study.

All cases except three showed antibodies to complement C3 emphasizing the role of antibody mediated complement deposition in the pathogenesis of pemphigus vulgaris as demonstrated by Joost and colleagues[56] and Jordan et al[28].

Two cases showed nonspecific findings on histopathology (Table-9), with DIF showing ICS pattern of staining. Both were from the perilesional skin of symptomatic patients clinically suspected to have pemphigus. The biopsy in both cases showed intact epidermis with mild dermal inflammation. There was also a separate strip of degenerate

epithelium lying detached from the skin in both cases. Since these two cases had given a strong positive reaction with DIF, the patients were started on treatment for pemphigus vulgaris, but subsequent follow up was lost. It is possible that histopathology was inconclusive in these cases due to either epidermal regeneration or because of a sampling error.

The other two cases, both from the oral mucosa, did not show acantholysis or any evidence of blister formation. One of them showed a strongly positive ICS staining (IgG, IgM and Fib were positive). Biopsy of this case showed focal mucosal ulceration, spongiosis, dense mixed inflammation of the lamina propria and focal lymphoid aggregates within a minor salivary gland lobule, suggestive but not diagnostic of chelitis glandularis (fig.5). This patient had only oral erosions with no skin lesions. The oral mucosa of the other patient, who also had erosions over both legs, showed features consistent with oral lichen planus (fig.4). In this patient, DIF showed weak ICS pattern of staining with IgG alone. Fibrinogen was negative (linear deposits of fibrinogen along the basement membrane are usually seen in lichen planus[14]). Based on clinical and DIF findings both patients were started on treatment for pemphigus vulgaris, but were subsequently lost on follow-up.

Patients presenting with only oral lesions can be seen in the initial phase of the disease[38]. Under these circumstances, the diagnosis of pemphigus vulgaris becomes rather difficult as the transient picture of typical vesicles and bulla is often obscured by trauma and secondary infection, both of which can clinically and histologically result in a nonspecific picture. Maurer et al[38], in their study, describe two patients with oral lesions showing only a dense band-like lympho-histiocytic infiltrate within the lamina propria,

without classical histological features. Both cases were positive for DIF with ICS pattern. This was similar to one of our cases. Hence in the absence of the characteristic histopathological features, which could be due to various causes, DIF becomes an important modality in the diagnosis of pemphigus vulgaris.

BULLOUS PEMPHIGOID

The second most common lesion observed in our study group was bullous pemphigoid (table-3) (13/40 cases, 32.5%) with the mean age of presentation being 60 years. Only one patient was aged between 20 -30 years (Table-1). Although earlier studies[39] and literature from western countries[10,41] have shown bullous pemphigoid to be the most common autoimmune blistering disorder, in our study it was second to pemphigus vulgaris. A study conducted at St. John's hospital, Bangalore, India[20] also showed a similar pattern with a higher number of cases with pemphigus vulgaris. The affected patients were predominantly females (84.6%). DIF (Table-7) was strongly positive in 10/13 (76.9%) cases. All positive cases showed linear staining in the Basement Membrane Zone (BMZ) (fig.12). The deposits were of homogenous linear type. Complement component C3 was present in all 12 positive cases (fig.13), in combination with IgG. Studies by Jordan et al[46] and Chorzelski and Carmone[46] have shown that C3 was present in almost every single case of bullous pemphigoid when compared to pemphigus vulgaris, where a minority of cases was invariably negative. IgA or IgM were present in two cases along with IgG and C3. One case was negative for DIF.

Correlation between DIF and histopathology was high (Table-8) with most cases showing a subepidermal bulla (11/13). Eosinophils (8/13) were the predominant inflammatory cell (fig.9). Two cases (Table-5) showed a sparse dermal inflammatory

infiltrate (cell–poor pattern) (fig.10).

The case which was negative on DIF had a sub-epidermal bulla with eosinophils and neutrophils in the dermis. The patient had active disease and the clinical differential diagnoses had included dermatitis herpetiformis and bullous pemphigoid. Since histopathology was more in favour of bullous pemphigoid and the patient was not willing for a repeat biopsy for immunofluorescence, she was started on therapy for bullous pemphigoid. She was advised for a repeat biopsy for DIF, but was lost on follow up.

There was one case (Table-9) which showed only dermis on histopathology. There was a mild perivascular lymphocytic infiltrate in the superficial dermis. DIF from peri-lesional skin showed a moderately strong linear BMZ pattern of staining with IgG and C3. Loss of the epidermis, which was probably the roof of the blister, could have occured during biopsy or in histoprocessing. A repeat sample was advised but the patient, having been started on treatment for bullous pemphigoid, was lost on follow-up.

PEMPHIGUS FOLIACEOUS

Of the four cases of pemphigus foliaceous, three cases were between the ages of 51 and 70 (Table-1). One case was 28 years old. The age group affected by foliaceous is usually quite variable[10]. There was equal distribution of the disease between both sexes which was in concordance with earlier studies[47].

All four cases showed ICS pattern of staining, of which three had staining restricted to the upper 2/3rd of the epidermis (Table-7) (fig.17). The fourth case had full thickness ICS pattern of staining. The latter along with 2 other cases showed a sub-corneal blister on histopathology (Table-6).

One case showed discordant histopathological findings (Table-9). This patient was a 52 year old male who was sick and had generalized pustular lesions. The clinical differential diagnoses included pustular psoriasis and pemphigus foliaceous. Histopathology showed epidermal spongiosis and subcorneal collections of neutrophils with no evidence of sub-corneal bulla, cleft or acantholytic cells (fig.16). A diagnosis compatible with pustular psoriasis was suggested. DIF showed a moderately strong ICS pattern of staining with IgG alone which was restricted to upper 2/3rd of the epidermis. Due to the discordant findings and the need for rapid therapeutic intervention, the patient was started empirically on both methotrexate and omnacortil (treated for both pustular psoriasis and pemphigus foliaceous). The lesions subsided. Immunoblotting and ELISA were subsequently done on blood samples sent to the Dept of Immunodermatology, St. Thomas Hospital in London. Both tests were positive for anti-desmoglein1 (fig.28). This is diagnostic of pemphigus foliaceous. The patient is presently on treatment for pemphigus foliaceous alone with no new lesions and hence a repeat biopsy for histopathology could not be done. Biopsies from patients with pemphigus foliaceous may occasionally show only collections of neutrophils in the subcorneal plane and hence may mimic subcorneal pustular lesions like pustular psoriasis[10]. The findings in DIF proved invaluable in this case as both clinical and histological findings had been misleading.

PEMPHIGUS VEGETANS:

We had one patient, a 34 year old female who had presented with pustular lesions which broke down to form erosions over the axillary skin and oral mucosa. She then developed vegetating lesions over both axillae.

Histopathology showed a supra-basal blister with epidermis exhibiting hyperkeratosis,

marked acanthosis and papillomatosis (fig.18, 19). Acantholysis was seen extending into to follicular epithelium. DIF showed moderately strong ICS staining pattern with IgG and C3 involving the entire thickness of the epidermis (Table-7) (fig. 20).

Pemphigus vegetans is a chronic clinical variant of pemphigus vulgaris and carries a better prognosis. In addition to the clinical findings, histopathology may show a few characteristic features such as marked acanthosis, pseudoepitheliomatous hyperplasia and multiple epidermal microabsesses which distinguish it from pemphigus vulgaris[10,41]. DIF shows ICS pattern of staining which complements the diagnosis. DIF (with the five antibodies that we have used in our study) cannot be used as a 'gold standard' in the diagnosis of pemphigus vegetans because as a stand-alone investigation, it cannot differentiate between pemphigus vulgaris and pemphigus vegetans.

CHRONIC BULLOUS DERMATOSIS OF CHILDHOOD

There were two cases of chronic bullous dermatosis of childhood – a boy and a girl aged 6 and 7 years respectively (Table-1). Both patients had characteristic clinical features such as an annular pattern of distribution of the vesicles, pruritus and post-inflammatory pigmentation. Histopathology of both cases showed sub-epidermal bullae with a neutrophil rich dermal inflammatory infiltrate (fig. 21, 22).

DIF (Table-7) of both cases showed moderately strong homogenous linear pattern of staining along the basement membrane zone with IgA (fig.23). One of the cases, in addition to IgA, showed focal linear BMZ staining with IgG and IgM. Studies by Leonard et al[32] have shown the significance of differentiating homogenous linear BMZ deposits of IgA from granular linear deposit, as the latter would be more in favour of dermatitis herpetiformis.

The occurrence of a weak IgG along with IgA in one of the patients did raise childhood bullous pemphigoid as a possibility since they too can present with linear IgA deposits. Apart from the clinical and histological features - which were in favour of chronic bullous disease of childhood - the staining intensity of the IgA deposits was much stronger than that of IgG. In addition, C 3 was negative. Both these factors, according to studies done by Beutner et al[14], are more suggestive of chronic bullous dermatoses of childhood, in the absence of characteristic clinical findings.

BULLOUS SLE:

This patient was a 46 year old female who had presented with pruritic raised lesions over the extensor aspect of both upper extremities. Some of these lesions developed into blisters. The patient also gave history of photosensitivity. Investigation done for anti-nuclear antibody (ANA) was negative.

Histopathology showed a subepidermal vesicle and a lymphocyte predominant perivascular inflammatory cell infiltrate with evidence of mild vasculitis within the superficial dermis (fig.24, 25). Alcian blue with periodic acid Schiff (PAS) showed mild increase in dermal mucin. DIF showed moderately strong granular BMZ pattern of staining with both IgG (fig.26) and C3 (fig. 27). Granular BMZ staining can be seen in upto 40 % cases when compared to linear deposits[10]. Although a salt-split skin with either DIF or IDIF or immunoblotting is necessary for excluding bullous pemphigoid[44], the history of photosensitivity could not be ignored. Patient was put on dapsone to which she responded well and did not develop any further lesions. The patient was not willing for further investigations.

SUMMARY

The proportion of immunobullous diseases out of the total number of skin biopsies received by our institution over a period of two years was 82. Of these 82 cases, we included only those cases for which both direct immunofluorescence and histopathology had been done (total 40 cases). The remaining 42 cases had either only histopathology or immunofluorescence done and hence were not included.

The correlation between histopathology and DIF was high in our study and comparable to previous studies[20, 30], but the study is limited by the fact that our sample size was small. There were two false negative cases (both patients had clinically active disease) which could have probably been due to technical or sampling errors.

ROLE OF DIRECT IMMUNOFLOURESCENCE:

Immunoflourescence studies have played a vital role in the diagnosis, and more importantly, in understanding the pathophysiology of vesicobullous lesions of the skin[20]. Although the technique has developed rapidly in western countries, its use as a diagnostic tool remains considerably low in our country. Two primary reasons that can be attributed to this are high cost and technical expertise. Although DIF has been shown in the past to be of prognostic value, this view remains highly contested[22, 44].

Although we found a high correlation between DIF and histopathology in our study, the role of DIF was highlighted especially in those cases where histopathology was inconclusive or not compatible with the clinical diagnosis. This is particularly true in subepidermal blistering diseases where overlapping clinical and histological features cause a common diagnostic difficulty. DIF differentiates these disorders based on the type of auto-antibody present and the pattern of staining of the BMZ (Basement Membrane Zone).

Further studies using salt-split skin with direct or indirect immunofluorescence and immunoblotting may be needed to differentiate diseases like bullous pemphigoid and epidermolysis bullosa aquisita, due to similar staining patterns on DIF.

The utility of DIF in the diagnosis of immunobullous lesions is unquestionable. However, in resource poor settings such as India, it is not economically feasible to perform immunofluorescence studies on all patients. The high correlation obtained in our study also indicates that histopathology was accurate in most of the cases. In these cases DIF played a supplementary role in the diagnostic process. On the other hand, DIF findings were crucial in the diagnosis of lesions with confounding clinical and histopathological features. DIF, histopathology and clinical features form a triad of diagnostic tools which complement each other. However, if and when there is a need for a cost-effective solution, DIF can be used selectively in lesions belonging to the 'grey-zone', which include cases with clinical variations or those with inconclusive histopathological features or both.

Immunobullous lesions carry a grave prognosis and an accurate diagnosis with early therapeutic intervention is crucial in reducing the morbidity of the patient. Clinical and histopathological features along with the judicial use of immunofluorescence techniques together provide cost-effective but accurate diagnosis.

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LIST OF ABBREVIATIONS

1) HPE - HISTOPATHOLOGY
2) DIF - DIRECT IMMUNOFLUORESCENCE
3) PV - PEMPHIGUS VULGARIS
4) BP - BULLOUS PEMPHIGOID
5) PF - PEMPHIGUS FOLIACEOUS
6) VEG – PEMPHIGUS VEGETANS
7) CBDC- CHRONIC BULLOUS DERMATOSIS OF CHILDHOOD
8) SLE – SYSTEMIC LUPUS ERYTHEMATOSUS
9) ICS – INTERCELLULAR SUBSTANCE
10) BMZ – BASEMENT MEMBRANE ZONE
11) IgG – IMMUNOGLOBULIN G
12) IgA – IMMUNOGLOBULIN A
13) IgM – IMMUNOGLOBULIN M
14) C 3 – COMPLEMENT COMPONENT OF THE ALTERNATE PATHWAY
15) FIB - FIBRINOGEN